

Detection of Von Willebrand Factor (Factor 8) in Formalin Fixed, Paraffin-Embedded Rodent Tissue

Reagents:

[1X Automation Buffer](#)
[3% Hydrogen Peroxide](#)
[Antibody Diluent](#)
[Citrate Buffer](#)
[DAB Chromagen](#)
[Hematoxylin](#)

Antibody Information:

Kit: Rabbit Elite kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com

1-800-227-6666

Catalog: PK-6101

*The Vector Rabbit Elite Kit contains solutions needed to make the secondary and label antibodies.

Avidin Biotin Blocking Kit

Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com

1-800-227-6666

Catalog #SP-2001

Retrieval Reagent: Carenzym II (Pepsin)

Biocare Medical
Walnut Creek CA
www.biocare.net

1-800-799-9499

Catalog# pep956H

Primary antibody: Rabbit Anti-Human Von Willebrand Factor (Factor 8)

Biocare Medical

1-800-799-9499

Concentration: 1 mg/ml

Catalog #CP039A

Negative control serum: Normal Rabbit Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Concentration: 60 mg/ml

Catalog #011-000-001

Staining Procedure

-Positive Control Tissue: Endothelial cells

-Stain Localization: Cytoplasmic

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% EtOH	2 times	3 minutes
95% EtOH	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.

2. Rinse slides in one change of 1X Automation Buffer for 5 minutes at room temperature.

3. Place slides in one change of 1X Automation Buffer for 5 minutes at 37 degrees.

4. Place slides on rack at 37 degrees.

Apply ready-to-use Carezyme-II solution to slides for 5 minutes. (Carezyme II should be at RT prior to use)

Comment: Heat-induced epitope retrieval does not work well with this antibody. Enzyme treatment is the preferred method of antigen retrieval.

5. Rinse slides in distilled water for 1 minute.

6. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes.

7. Apply blocking solution from the Vector Rabbit Elite kit and incubate for 20 minutes.

Exp. Date _____ New Kit yes / no

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

8. Apply the Avidin Biotin Blocking kit

Lot# _____ Exp Date _____ New Kit yes / no

Apply avidin block - 15 min @ RT.

Quick rinse in 1X Automation Buffer. (Do not leave slides sitting in buffer)

Apply biotin block - 15 min @ RT.

No wash, wipe excess block and apply primary antibody

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

9. Apply primary antibody (Rabbit anti-Human Factor 8) at a 1:300 dilution and incubate one hour.

Lot# _____ Exp Date _____

For negative control slides, normalize the protein concentration of the normal rabbit serum to the protein concentration of the primary antibody (Factor8) and use this to make 1:300 the dilution and incubate for one hour.

Lot# _____ Reconstituted Date _____

10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

11. Apply secondary antibody from the Rabbit Elite Kit and incubate for 30 minutes.

12. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

13. Apply Label antibody from Vector Rabbit Elite Kit and incubate for 30 minutes.
(Prepare at least 30 mins prior to use)

14. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each

15. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
(Add 1 drop of DAB per ml of substrate)

Lot# _____ Exp. Date _____ New Kit yes / no

16. Rinse in tap water 3 minutes.

17. Counterstain with Modified Harris Hematoxylin for 30 seconds.

18. Rinse in tap water until clear.

19. Place slides in 1X Automation buffer for 1 minute with gentle agitation to blue slides.

20. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% EtOH	3 changes	3 minutes
Xylene	2 changes	5 minutes

21. Coverslip

updated 2/13/04